Biocontrol capabilities of the genus *Serratia*

Amalia Soenens¹ and Juan Imperial^{1,2*}

¹ Centro de Biotecnología y Genómica de Plantas (UPM-INIA) and

² Instituto de Ciencias Agrarias (CSIC), 28006 Madrid, Spain

*Corresponding author's address: Instituto de Ciencias Agrarias (CSIC), Serrano 115 bis, 28006 Madrid, Spain. Email: juan.imperial@csic.es

Abstract

This review focuses on the production of natural bioactive products and their biocontrol capabilities of the enterobacterial genus *Serratia*. *Serratia* represents a unique group of enterobacteria with a notable secondary metabolism, able to produce a wide range of natural bioactive products including the β -lactam antibiotic carbapenem or the antifungal compound oocydin A. However, until very recently, most of the *Serratia* isolates originated from human and animal infections, and a systematic approach to the isolation of *Serratia* from natural habitats has been missing. The paucity of environmental isolates has so far limited our understanding of the potential of *Serratia* to produce new natural bioactive products and their capacity to be used in sustainable agriculture as biocontrol agents. The ability to isolate *Serratia* from soils, together with the analytical capabilities

afforded by the democratization of genome sequencing methodologies, opens the door to the isolation and characterization of such novel biocontrol agents, hitherto inaccessible.

Introduction

In 1823, Bartolomeo Bizio, a pharmacist from Padua in Italy, described *Serratia marcescens* (Bizio 1823) representing the type species of the new genus. A remarkable characteristic of this bacterium was the production of a bright-red pigment called prodigiosin (Williams 1973). A miraculous bloody discolouration in polenta (corn mush) and bread drew the attention of Bizio. He identified a microorganism as the cause of this phenomenon that he named *Serratia marcescens*: *Serratia* in honour of an Italian physicist named Serafino Serrati, and *marcescens* from the Latin word for decaying, because the blood pigment was found to deteriorate quickly. The pigment responsible for the bloody discolouration was later on described and named prodigiosin. Members of the type species *S. marcescens* are prodigiosin producers and opportunistic nosocomial pathogens, and represent the most important human pathogens within the genus (Eisenstein 1990).

Bacteria from the genus *Serratia* are Gram-negative bacteria from the Enterobacteriaceae family that are classified, to date, in eighteen described species. It is an extremely diverse group of bacteria capable of occupying many different habitats such as water, soil, plants, vertebrates and humans, that includes biologically and ecologically diverse species, from those that are beneficial to economically important plants, to pathogenic species that are harmful to humans (Grimont 1984).

Although only a few *Serratia* isolates have been characterised in detail, a noteworthy characteristic of these bacteria is their secondary metabolism and their ability to produce a wide range of natural bioactive products (Slater et al. 2003; Kai et al. 2007; Kai et al. 2010; Matilla et al. 2012; Matilla et al. 2015, Domik et al. 2016b), including the red-pink, non-diffusible pigment, prodigiosin.

***Serratia* as producers of natural bioactive molecules**

Prodigiosin

Production of prodigiosin (2-methyl-3-pentyl-6-methoxyprodiginine) is the distinctive characteristic of the genus. Prodigiosin is a tripyrrole red-pigment, bioactive molecule from the family of prodiginines and it is secreted to the culture medium as a secondary metabolite (Slater et al. 2003). The production of prodigiosin is restricted to four species: *S. marcescens*, *S. plymuthica*, *S. nematodiphila* and *S. rubidaea* (Grimont 1984). It is produced in the late exponential phase of growth (Slater et al. 2003) and has been shown to be influenced by numerous environmental factors, including phosphate availability, media composition, temperature and pH (Slater et al. 2003; Williams 1980; Solé et al. 1994; Williams et al. 1971). The ability to produce prodiginines has been also described for other groups of bacteria, such as actinomycetes (*Streptomyces coelicolor* A3(2) and *S. griseovirides*), and marine bacteria, such as *Hahella chejuensis* KCTC 2396 and *Pseudoalteromonas denitrificans* (Perry 1961; Kawauchi et al. 1997; Jeong et al. 2005).

This group of compounds has received special attention in view of its numerous bioactivities. Prodigiosin has long been a subject of research interest due to its many potential beneficial properties such as antibacterial, antiprotozoal, antimalarial, anticancer and immunosuppressive activities (Williams 1980; Pérez-Tomás et al. 2003; Demain 1995; Bennett and Bentley 2000; Hsieh et al. 2012; Espona-Fiedler et al. 2012; Lu et al. 2012). Furthermore, prior to the development of synthetic colour chemistry, *S. marcescens* prodigiosin was produced commercially, for the dyeing of silk and wool (Yusof 2008).

In *Serratia*, the prodigiosin biosynthetic gene cluster (*pig* cluster) is comprised of 14 tightly clustered genes, *pigABCDEFGHIJKLMN*, that span a continuous stretch of 20,960 bp (Harris et al. 2004). Prodigiosin biosynthesis has been shown to be under quorum sensing control in *Serratia* (Thomson et al. 2000).

The *in vivo* physiological role of bacterial prodigiosin remains undefined. A number of reports have suggested potential functions that may provide an advantage in competition with other organisms (Gulani et al. 2012) and against the continuously challenging natural environment (Stankovic et al. 2014).

The antibacterial properties of prodigiosin have been widely studied. The main target site for prodigiosin is the bacterial plasma membrane. As a chaotropic stressor, prodigiosin disrupts the bacterial plasma membrane and induces loss of essential intracellular substances such as sugars, amino acids, proteins and K^+ ions from prodigiosin-treated bacteria (Suryawanshi et al. 2017). Prodigiosin has been demonstrated to exhibit antagonistic effects towards *Staphylococcus aureus*, *Bacillus cereus* (Gulani et al. 2012), *Acinetobacter anitratus*, *Agrobacterium tumefaciens*, *Bacillus licheniformis*, *B. subtilis*,

B. thuringiensis, *Erwinia* sp., *Escherichia coli*, *Micrococcus* sp., Methicillin-resistant *Staphylococcus aureus* (MRSA), *S. epidermidis*, *S. saprophyticus* (Ibrahim et al. 2014), *Streptococcus pyogenes*, *Enterobacter faecalis* and oxacillin-resistant *S. aureus* (ORSA) (Lapenda et al. 2015). Collectively, these findings show that prodigiosin is a potential broad-spectrum antibacterial agent.

Carbapenem

Serratia isolates also produce antibiotics of the carbapenem group. Carbapenems constitute a diverse group of β -lactam antibiotics characterised by potent antibacterial and β -lactamase-inhibitory activity. They have a broad spectrum and have important applications as antimicrobial agents, particularly in infections mediated by multidrug-resistance bacteria (Moellering et al. 1989). *Serratia* make a simple carbapenem, 1-carbapen-2-em-3-carboxylic acid (systematic name (R)-7-oxo-1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid; McGowan et al. 1996). Production and regulation of carbapenem has been widely studied in *S. marcescens* and *Serratia* sp. ATCC 39006. In *Serratia*, the *car* cluster involved in the production of the antibiotic is composed of nine genes, *carRABCDEFGH* (McGowan et al. 1996). The *carA-E* genes encode biosynthetic enzymes, while *carF* and *carG* encode proteins involved in a novel β -lactam resistance mechanism (McGowan et al. 1998; Li et al. 2000). The CarR protein is a member of the LuxR family of transcriptional regulators (McGowan et al. 1995) and acts as a positive regulator of the *carA-H* cluster. Production of carbapenem in *Serratia* has been linked to quorum sensing. CarR activates transcription of the remaining genes of the *car* cluster in response to *N*-(3-oxohexanoyl)-1-homoserine lactone (OHHL) synthesized by the product of the unlinked *carI* gene (Thomson et al. 2000).

Antifungal natural products

Antifungal natural products have also been described in *Serratia*. In 1999 oocydin A, a chlorinated macrolide, was first isolated from the plant epiphytic strain *S. marcescens* MSU97 because of its strong bioactivity against plant pathogenic oomycetes (Srobel et al. 1999). Oocydin A is an anti-fungal natural product of the polyketide type. Polyketides represent an important class of bioactive natural products with a broad range of biological activities (Piel 2010; Till and Race 2014; Hertweck 2009). Besides its anti-oomycete activity, oocydin A has also been shown to have antitumor haterumalide activity (Matilla et al. 2012). Oocydin A has also been shown to be produced by *S. plymuthica* strains A153, 4Rx5 and 4Rx13 (Matilla et al. 2015). The *ooc* gene cluster responsible for its biosynthesis spans a region between 77 and 80 kb containing a total of 23 open reading frames. It encodes five multimodular polyketide synthase (PKS) proteins (OocJ, OocL, OocN, OocR, and OocS), a hydroxymethylglutaryl-CoA synthase cassette and three flavin-dependent tailoring enzymes. This complex, large transacyltransferase (AT) polyketide synthase is organized in three transcriptional units (Matilla et al. 2015; Matilla et al. 2012). The oocydin gene clusters of *S. plymuthica* A 153 and *S. plymuthica* 4Rx13 are 78.2 and 78.3 kb long, respectively. In *S. plymuthica* A153, oocydin A is regulated by quorum sensing (Matilla et al. 2015). In vitro antifungal activity against fungal pathogens has also been observed with other *S. plymuthica* strains (Berg 2000). However, the chemical nature of these antifungal products has not been ascertained.

Volatile Organic Compounds: Sodorifen

Another characteristic compound produced by this genus is the recently described Sodorifen. Sodorifen is an unusual unique volatile hydrocarbon (1,2,4,5,6,7,8-heptamethyl-3-methylenebicyclo[3.2.1]oct-6-ene) (von Reuss et al. 2010). This remarkable structure is composed of one five-member and one six-member ring, where every carbon atom of the skeleton is substituted with either a methyl or a methylene group. Sodorifen was discovered as a volatile organic compound (VOC) emitted by *S. plymuthica* 4Rx13 (Domik et al. 2016a; Kai et al. 2010) but it has also been found to be emitted by a few other *S. plymuthica* isolates (Weise et al. 2014). Volatile compounds are characterised by molecular weights of less than 300 Da, high vapour pressures, low boiling points and low polarities. Microbial volatile organic compounds (mVOCs) are usually secondary metabolites produced by bacteria, however, the ecological functions of these mVOCs are largely unknown. It is thought that they can act as signals for communication between various organisms or serve as attractant or defense compounds (Wenke et al. 2010; Effmert et al. 2012; Wenke et al. 2012).

Although the biological function of sodorifen remains elusive, it has recently been shown that it could derive from terpene metabolism, and the gene cluster responsible of its biosynthesis has been identified (Domik et al. 2016b). *S. plymuthica* PRI-2C for example, produced sodorifen when exposed to VOCs emitted by the fungal pathogen *Fusarium culmorum* (Schmidt et al. 2017). In many *S. plymuthica* strains, the sodorifen cluster is made up of four co-transcribed genes, *sodA–D*, resulting in an expected transcript with a total length of 4.5 kb. The sodorifen synthase gene *sodD* forms a cluster with three additional genes encoding a methyltransferase, *sodC*, a 1-deoxy-D-xylulose-5-phosphate

synthase, *sodB*, and an isopentenyl-diphosphate delta-isomerase, *sodA*. The cluster is bordered by genes that are in antisense orientation and likely unrelated (Domik et al. 2016b; Schmidt et al. 2017).

Other Secondary Metabolites

Serrawettins are biosurfactants produced by *Serratia* (Soberón-Chávez and Maier 2011). They are non-ionic, as they have no amino acid residues with ionic hydrophilicity (Matsuyama et al. 2011). Serrawettins have not been described outside the *Serratia* genus and are gaining increased scientific interest as they have been shown to display emulsification, surface, antifouling, antitumor and antimicrobial activities (Escobar-Díaz et al. 2005; Dusane et al. 2011; Nalini and Parthasarathi 2014; Su et al. 2016). They are also able to modify the hydrophobicity of the cell surface, which plays an important role in the adhesion of these bacteria to various surfaces and contributes to enhancing the surface spreading of bacteria in nutrient-poor environments (Bar-Ness et al. 1988; Su et al. 2016; Matsuyama et al. 2011).

Three molecular species, serrawettin W1, W2, and W3, have been reported. Serrawettin W1 is the best studied of all, while serrawettin W3 has only been partially characterised (Matsuyama et al. 1986). All serrawettins are produced by *S. marcescens* strains, but serrawettin W2 has also been found to be produced by *S. surfactantfaciens* sp. nov. YD25 (Matsuyama et al. 1985; Su et al. 2016; Matsuyama et al. 1990; Matsuyama et al. 1992). *S. rubidaea* produces two differentiated, characteristic variants, rubiwettin R1 [3-(3'-hydroxytetradecanoyloxy) decanoate and 3-(3'-hydroxyhexadecanoyloxy) decanoate]

and RG1 [β -D-glucopyranosyl 3-(3'-hydroxytetradecanoyloxy) decanoate minor fatty acid isomers] (Matsuyama et al. 1990; Clements et al. 2019).

Serrawettin W1 has been described as a potent anti-cancer drug, which was able to inhibit cell growth and induce apoptosis of several cell lines derived from T-cell leukemia or Burkitt lymphoma (Garding et al. 2013; Sun et al. 2015). Serrawettin W1 is identical to the serratamolide discovered previously (Wasserman et al. 1961, 1962) as an antibiotic produced by *S. marcescens*. The first serrawettin was isolated in 1985 from a *S. marcescens* strain (Matsuyama et al. 1985; Matsuyama et al. 1986). Serrawettin W1 has also been reported to have broad-range antimicrobial activity, while promoting swarming motility, a behaviour that may allow the bacterium to become less susceptible to antibiotics and to move into more favourable microenvironments (Matsuyama et al. 1989; Overhage et al. 2008; Lai et al. 2009; Kadouri and Shanks 2013; Lapenda et al. 2015; Danevčič et al. 2016; Suryawanshi et al. 2017). Serrawettin W2 was first isolated from *S. marcescens* in 1986 (Matsuyama et al. 1986) and is a biosurfactant that can disperse *Caenorhabditis elegans* (Pradel et al. 2007), and displays antimicrobial activity against *Staphylococcus aureus* (Gerc et al. 2014). Several *S. marcescens* strains (Matsuyama et al. 1986; Tanikawa et al. 2006; Soto-Cerrato et al. 2005; Soo et al. 2014) have been described to produce prodigiosin and serrawettin W1 at the same time. Recently it has been reported that *S. surfactantifaciens* YD25 can simultaneously produce both prodigiosin and serrawettin W2 (Su et al. 2016).

Another *Serratia* secondary metabolite is althiomycin. Althiomycin was discovered in the *S. marcescens* strain Db10, a non-pigmented model insect pathogen (Gerc et al. 2012). This diffusible metabolite was able to inhibit the growth of the gram-positive bacterium *Bacillus subtilis*. Althiomycin is a broad-spectrum antibiotic first isolated from

Streptomyces althioticus 1957 (Yamaguchi et al. 1957). It is a heavily modified pentapeptide that inhibits protein biosynthesis by blocking the action of the peptidyl transferase (Fujimoto et al. 1970; Burns and Cundliffe 1973). In *S. marcescens* Db10, althiomycin biosynthesis is directed by a six-gene operon (*alb1–alb6*) encoding a hybrid, non-ribosomal peptide synthetase-polyketide synthase (NRPS-PKS) assembly line (Alb4/5/6), two tailoring enzymes (Alb2/3) and an export/resistance protein (Alb1) (Gerc et al. 2012). The production of althiomycin may confer a significant competitive advantage for the survival of *S. marcescens* Db10 as it was seen that this antibiotic is produced under normal culture conditions despite the high energetic load of biosynthesizing the extremely large NRPS- PKS proteins (Gerc et al. 2012).

A structural representation of the above natural bioactive compounds is shown in Figure 1.

***Serratia* as biocontrol organisms**

Some living organisms have the capacity to limit the adverse effects of pathogens. This suppression of pathogenesis is known as biological control, or biocontrol, and promises to be a useful alternative approach in the control of plant pathogens within sustainable agriculture systems (Johansson et al. 2004; Bale et al. 2008). Many soil bacteria can produce antibacterial, antifungal or mVOC compounds able to inhibit pathogenic bacteria or fungi. In *Serratia*, as a consequence of their secondary metabolism, a few strains have been described as endowed with biocontrol capabilities (Table 1).

Secretion of effectors using T6SS

Besides producing biocontrol compounds, *Serratia* displays an additional strategy for competition in mixed bacterial populations through the secretion of effectors using the Type 6 Secretion System (T6SS). T6SS is a protein secretion system widely present in Gram-negative bacteria that contributes to the virulence of pathogenic bacteria. T6SS, in their different variants, allow the controlled release of specific cellular proteins across the cell envelope to the exterior of the bacterial cell (Murdoch et al. 2011). *S. marcescens* Db10 has been shown to use T6SS to deliver multiple anti-bacterial effectors, endowing it with potent activity against closely and distantly related competitors (Murdoch et al. 2011). Later on, it was described that the Rhs proteins, T6SS-dependant antibacterial toxins, are the primary determinants of intraspecies competition between *S. marcescens* Db10 and closely related organisms. One particular Rhs effector was described to have DNase activity, whereas another represented a novel, cytoplasmic-acting toxin (Alcoforado Diniz et al. 2015).

Some of the functional characteristics of T6SS from *S. marcescens* have been studied in detail. For instance, this syringe-like molecular machine does not depend on contact for firing. As a result, *S. marcescens* adopts an “aggressor” strategy, broadening the target range of its T6SS that can now kill prey bacteria with an anti-bacterial T6SS or other incursive strategy, as well as efficiently kill those without a T6SS (Murdoch et al. 2011). The ability to trigger such unprovoked attacks, broadens its target range and may enhance its fitness in highly competitive niches (Gerc et al. 2014). Finally, it has been recently described that the antibacterial T6SS from *S. marcescens* also displays antifungal activity. This represents the first report of T6SS-dependent antifungal effectors. The two described

effectors, Tfe1 and Tfe2, elicit distinct responses in fungal cells and can exert a fungicidal effect (Trunk et al. 2018).

New *Serratia* soil strains with biocontrol potential: *Serratia* sp. B1_6 as a biocontrol candidate

All of the above studies suggest that members of *Serratia* are endowed with a rich secondary metabolism and are able to produce a range of biologically active compounds as part of their adaptation to their ecological niche(s). This makes them interesting candidates for biocontrol strategies. However, the above discussion also shows a limitation of studies on the biocontrol potential of *Serratia* strains, that of the availability of isolates. Until now, most of the available strains have been isolated either serendipitously from natural habitats or have relied on their pathogenicity to human or other hosts. Also, many of the isolates are prodigiosin producers, and no doubt the bright-red colour of colonies has contributed to their being singled out. We have recently succeeded in optimizing an enrichment strategy that has resulted in the isolation of a large number of *Serratia* strains from soil (Soenens PhD 2018). Preliminary studies suggest that these isolates exhibit a large genetic diversity, thus opening the way to the characterization of novel *Serratia* capabilities, especially regarding production of bioactive compounds and biocontrol capabilities.

As a proof-of-concept of such a strategy, we carried out a broad genomic characterization of one of these isolates, *Serratia* sp. B1_6 (Soenens PhD 2018), both genomically and regarding biocontrol potential. Analysis of the high-quality draft genomic sequence of this strain (Table 2; our unpublished results), suggest that strain B1_6 is closely related

to *S. plymuthica* 4Rx13 (Average nucleotide identity of 99.4), one of the best studied strains regarding biocontrol potential (Kai et al. 2010; Weise et al. 2014; Domik et al. 2016b). Preliminary screenings suggest that strain B1_6 exhibits a noteworthy capacity for production of antibiotics and antifungals (Figure 2). Bioinformatic scanning of the strain B1_6 genome sequence for the presence of known or inferred biosynthetic clusters for production of bioactive compounds (antiSMASH server, (Weber et al. 2015)) resulted in the identification of a large number of clusters similar to known antibiotic, antifungal and mVOCs biosynthetic clusters (Table 3). Notably, *Serratia* sp. B1_6 contained biosynthetic clusters for oocydin A and sodorifen. Antifungal activity has been shown to be common in numerous examples of *Serratia* strains used or described as biocontrol agents (Kurze et al. 2001; Grosch et al. 2005; Berg 2000). The ability of *Serratia* sp. B1_6 to produce oocydin A could explain its ability for biocontrol against the plant pathogen *Verticillium dahliae* (Figure 2). However, the remarkably high antifungal activity obtained could be taken as indication of the possible production of more than one antifungal compounds. Sodorifen, an intriguing mVOC characteristic of *Serratia*, with a unique structure, and whose biological function is still unknown, was predicted in the *Serratia* sp. B1_6 genome. Sodorifen has been shown to originate from terpene metabolism (see above), and terpene biosynthetic capabilities were also predicted in the antiSMASH analyses for this strain.

Surprisingly, and despite the observed antibiotic activity (Figure 2), *Serratia* sp. B1_6 did not contain the *car* cluster responsible for carbapenem production, suggesting that a different type of antibiotic is produced by the strain. Given that carbapenems are β -lactam antibiotics (Thomson et al. 2000) and that a β -lactam sensitive strain (*E. coli* ESS) was used for screening, it is reasonable to expect the new antibiotics to be β -lactams; however,

the *E. coli* ESS indicator strain is also sensitive to other antibiotics (Nishida et al. 1977). A final possibility is that *Serratia* sp. B1_6 genome produces carbapenem with a completely different set of genes that do not display any sequence similarity to the known *car* clusters. In any case, further characterisation of the antibiotics produced should be carried out. Chemical strategies for such a characterisation are classical nowadays (Talarico and Dobrogosz 1989; Dizman et al. 2005; Lambert et al. 2007; Niveshika et al. 2016).

Conclusions, challenges and future perspectives

Characteristically among *Enterobacteriaceae*, members of the genus *Serratia* display a rich secondary metabolism. Many of these secondary metabolites have biological activity and make of *Serratia* an attractive organism for biocontrol purposes. These properties appear to be the result of the particular lifestyle that these organisms have evolved in their different niches. This can be exemplified using the soil isolates, of which *Serratia* sp. B1_6 is a representative case. The soil is a complex and highly competitive environment, and different soil bacteria adopt different strategies for adaptation to these conditions. Some of them, such as *Pseudomonas*, display unsurpassed catabolic activities, and survive and thrive by outcompeting other organisms in their ability to: i) utilize a large variety of, often recalcitrant, carbon compounds, and ii) multiply efficiently on those substrates, giving rise to large populations in a short time span (Varivarn et al. 2013). Other organisms display particular adaptations to the rhizosphere of plants, sometimes with high specificity, such as with symbiotic rhizobia and legumes (Masson-Boivin et al. 2009). In those cases, the plant root provides a specialized environment, rich in secreted

or leaked plant metabolites (Hassan and Mathesius 2012). However, the most common strategy among native soil bacteria is that of “chemical warfare” (Czárán et al. 2002), with the synthesis and secretion of bioactive, inhibitory compounds to control possible competitors (Hibbing et al. 2010). This strategy is exemplified by the Actinobacteria, a large group of soil inhabitants wherefrom the majority of currently known antibiotics have been isolated (Baltz 2008; Newman and Cragg 2007; Bérdy 2012). *Serratia* isolates, notably those originating from soil (Soenens PhD 2018) appear to have adopted such a strategy. Our recent acquired ability to selectively isolate members of the genus *Serratia* from soil environments, together with the relatively simple and affordable ability to genomically characterize such isolates, opens a new avenue of research into the secondary metabolic capabilities of this genus, that could result in the characterization of new bioactive compounds potentially useful for biological control strategies.

Acknowledgements

We thank George P. C Salmond and Rita Monson from the Biochemistry Department at the University of Cambridge for their help and support in the *Serratia* phenotypic experiments and for all their knowledge sharing in conducting such experiments. AS is also grateful to the Consejo Social of the Universidad Politécnica de Madrid for a scholarship for Internationalization of PhD students.

Bibliography

- Alcoforado Diniz J, Liu Y, and Coulthurst S (2015) Molecular weaponry: diverse effectors delivered by the Type VI secretion system. *Cell Microbiol* 17 (12):1742-51
- Alström S (2001) Characteristics of Bacteria from Oilseed Rape in Relation to their Biocontrol Activity against *Verticillium dahliae*. *J Phytopathol* 149 (2):57-64
- Bale J, van Lenteren J, and Bigler F (2008) Biological control and sustainable food production. *Philos Trans R Soc Lond B Biol Sci* 363 (1492):761-76
- Baltz R (2008) Renaissance in antibacterial discovery from actinomycetes. *Curr Opin Pharmacol* 8 (5):557-63
- Bar-Ness R, Avrahamy N, Matsuyama T et al. (1989) Increased cell surface hydrophobicity of a *Serratia marcescens* NS 38 mutant lacking wetting activity. *J Bacteriol* 170 (9):4361-4
- Bennett J and Bentley R (2000) Seeing red: the story of prodigiosin. *Adv Appl Microbiol* 47:1-32
- Berg G (2000) Diversity of antifungal and plant-associated *Serratia plymuthica* strains. *J Appl Microbiol* 88 (6):952-60
- Bizio B (1823) Lettera di Bartolomeo Bizio al chiarissimo canonico Angelo Bellani sopra il fenomeno della polenta porporina. *Biblioteca Italiana o sia Giornale di Letteratura, Scienze e Arti* (Anno VIII)
- Burns D and Cundliffe E (1973) Bacterial-protein synthesis. A novel system for studying antibiotic action in vivo. *Eur J Biochem* 37 (3):570-4
- Bérdy J (2012) Thoughts and facts about antibiotics: where we are now and where we are heading. *J Antibiot (Tokyo)* 65 (8):385-95

- Clements T, Ndlovu T, Khan S and Khan W (2019) Biosurfactants produced by *Serratia* species: Classification, biosynthesis, production and application. Appl Microbiol Biotechnol 103 (2):589-602.
- Czárán T, Hoekstra R and Pagie L (2002) Chemical warfare between microbes promotes biodiversity. Proc Natl Acad Sci U S A 99 (2):786-90
- Danevčič T, Borić Vezjak M, Tabor M et al. (2016) Prodigiosin Induces Autolysins in Actively Grown *Bacillus subtilis* Cells. Front Microbiol 7:27
- Demain A (1995) Why do microorganisms produce antimicrobials? In Fifty Years of Antimicrobials: Past Perspectives and Future Trends (Society for General Microbiology Symposium no. 53). Edited by P. A. Hunter, G. K. Darby & N.J. Russell. Cambridge: Cambridge University Press
- Dizman B, Elasri MO and Mathias LJ (2005) Synthesis, characterization, and antibacterial activities of novel methacrylate polymers containing norfloxacin. Biomacromolecules 6 (1):514-20
- Domik D, Magnus N and Piechulla B (2016b) Analysis of a new cluster of genes involved in the synthesis of the unique volatile organic compound sodorifen of *Serratia plymuthica* 4Rx13. FEMS Microbiol Lett 363 (14):fnw139
- Domik D, Thurmer A, Weise T et al. (2016a) A Terpene Synthase Is Involved in the Synthesis of the Volatile Organic Compound Sodorifen of *Serratia plymuthica* 4Rx13. Front Microbiol 7:737
- Dusane D, Pawar V, Nanchaiaiah Y et al. (2011) Anti-biofilm potential of a glycolipid surfactant produced by a tropical marine strain of *Serratia marcescens*. Biofouling 27 (6):645-54
- Effmert U, Kalderas J, Warnke R et al. (2012) Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol 38 (6):665-703

- Eisenstein I (1990) Enterobacteriaceae. Churchill Livingstone New York: In: Mandell GL, Douglas RG, Bennett JE, Eds. Principles and Practice Of Infectious Disease 3rd ed
- Escobar-Díaz E, López-Martín E, Hernández del Cerro M et al. (2005) AT514, a cyclic depsipeptide from *Serratia marcescens*, induces apoptosis of B-chronic lymphocytic leukemia cells: interference with the Akt/NF-kappaB survival pathway. *Leukemia* 19 (4):572-9
- Espona-Fiedler M, Soto-Cerrato V, Hosseini A et al. (2012) Identification of dual mTORC1 and mTORC2 inhibitors in melanoma cells: prodigiosin vs. obatoclax. *Biochem Pharmacol* 83 (4):489-96
- Fujimoto H, Kinoshita T, Suzuki H et al. (1970) Studies on the mode of action of althiomycin. *J Antibiot (Tokyo)* 23 (6):271-5
- Fürnkranz M, Adam E, Müller H et al. (2012) Promotion of growth, health and stress tolerance of Styrian oil pumpkins by bacterial endophytes. *Eur J Plant Pathol* 134 (3):509-19
- Garding A, Bhattacharya N, Claus R et al. (2013) Epigenetic upregulation of lncRNAs at 13q14.3 in leukemia is linked to the In Cis downregulation of a gene cluster that targets NF-kB. *PLoS Genet* 9 (4):e1003373
- Gerc A, Song L, Challis G et al. (2012) The insect pathogen *Serratia marcescens* Db10 uses a hybrid non-ribosomal peptide synthetase-polyketide synthase to produce the antibiotic althiomycin. *PLoS One* 7 (9):e44673
- Gerc A, Stanley-Wall N and Coulthurst S (2014) Role of the phosphopantetheinyltransferase enzyme, PswP, in the biosynthesis of antimicrobial secondary metabolites by *Serratia marcescens* Db10. *Microbiology* 160 (Pt 8):1609-17

- Grimont P and Grimont F (1984) Genus *Serratia* Bizio 1823, 288^{AL}. Baltimore: In: Krieg, N. R., and Holt, J.G. (ed.), Bergey's manual of systematic bacteriology. Williams and Wilkins
- Grosch R, Faltin F, Lottmann J et al. (2005) Effectiveness of 3 antagonistic bacterial isolates to control *Rhizoctonia solani* Kuhn on lettuce and potato. Can J Microbiol 51 (4):345-53
- Gulani C, Bhattacharya S and Das A (2012) Assessment of Process Parameters Influencing the Enhanced Production of Prodigiosin from *Serratia marcescens* and Evaluation of Its Antimicrobial, Antioxidant and Dyeing Potentials. Malays J Microbiol 8(2):116-22
- Harris A, Williamson N, Slater H et al. (2004) The *Serratia* gene cluster encoding biosynthesis of the red antibiotic, prodigiosin, shows species- and strain-dependent genome context variation. Microbiology 150 (Pt 11):3547-60
- Hassan S and Mathesius U (2012) The role of flavonoids in root–rhizosphere signalling: opportunities and challenges for improving plant–microbe interactions. J Exp Bot 63 (9):3429-44
- Hertweck C (2009) The biosynthetic logic of polyketide diversity. Angew Chem Int Ed Engl 48 (26):4688-716
- Hibbing M, Fuqua C, Parsek M et al. (2010) Bacterial competition: surviving and thriving in the microbial jungle. Nat Rev Microbiol 8 (1):15-25
- Hsieh H, Shieh J, Chen C et al. (2012) Prodigiosin down-regulates SKP2 to induce p27(KIP1) stabilization and antiproliferation in human lung adenocarcinoma cells. Br J Pharmacol 166 (7):2095-108

- Ibrahim D, Nazari T, Kassim J et al. (2014) Prodigiosin- an antibacterial red pigment produced by *Serratia marcescens* IBRL USM 84 associated with a marine sponge *Xestospongia testudinaria*. J Appl Pharm Sci 4 (10): 1-6
- Jeong H, Yim J, Lee C et al. (2005) Genomic blueprint of *Hahella chejuensis*, a marine microbe producing an algicidal agent. Nucleic Acids Res 33 (22):7066-73
- Johansson J, Paul L and Finlay R (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiol Ecol 48 (1):1-13
- Kadouri D and Shanks R (2013) Identification of a methicillin-resistant *Staphylococcus aureus* inhibitory compound isolated from *Serratia marcescens*. Res Microbiol 164 (8):821-6
- Kai M, Crespo E, Cristescu S et al. (2010) *Serratia odorifera*: analysis of volatile emission and biological impact of volatile compounds on *Arabidopsis thaliana*. Appl Microbiol Biotechnol 88 (4):965-76
- Kai M, Effmert U, Berg G et al. (2007) Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen *Rhizoctonia solani*. Arch Microbiol 187 (5):351-60
- Kawauchi K, Shibutani K, Yagisawa H et al. (1997) A possible immunosuppressant, cycloprodigiosin hydrochloride, obtained from *Pseudoalteromonas denitrificans*. Biochem Biophys Res Commun 237 (3):543-7
- Kurze S, Bahl H, Dahl R et al. (2001) Biological Control of Fungal Strawberry Diseases by *Serratia plymuthica* HRO-C48. Plant Dis 85 (5):529-34
- Lai S, Tremblay J and Déziel E (2009) Swarming motility: a multicellular behaviour conferring antimicrobial resistance. Environ Microbiol 11 (1):126-36
- Lambert M, Jean-Luc W, Stärk D et al. (2007) Identification of Natural Products Using HPLC-SPE Combined with CapNMR. Anal Chem 79 (2):727-35

- Lapenda J, Silva P, Vicalvi M et al. (2015) Antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEDA 398. World J Microbiol Biotechnol 31 (2):399-406
- Lavania M, Chauhan P, Chauhan S et al. (2006) Induction of plant defense enzymes and phenolics by treatment with plant growth-promoting rhizobacteria *Serratia marcescens* NBRI1213. Curr Microbiol 52 (5):363-8
- Li R, Stapon A, Blanchfield J et al. (2000) Three Unusual Reactions Mediate Carbapenem and Carbapenam Biosynthesis. J Am Chem Soc 122 (38):9296-7
- Lu C, Lin S, Yang S et al. (2012) Prodigiosin-induced cytotoxicity involves RAD51 down-regulation through the JNK and p38 MAPK pathways in human breast carcinoma cell lines. Toxicol Lett 212 (1):83-9
- Masson-Boivin C, Giraud E, Perret X et al. (2009) Establishing nitrogen-fixing symbiosis with legumes: how many rhizobium recipes? Trends Microbiol 17 (10):458-66
- Matilla M, Leeper F and Salmond G (2015) Biosynthesis of the antifungal haterumalide, oocydin A, in *Serratia*, and its regulation by quorum sensing, RpoS and Hfq. Environ Microbiol 17 (8):2993-3008
- Matilla M, Stockmann H, Leeper F et al. (2012) Bacterial biosynthetic gene clusters encoding the anti-cancer haterumalide class of molecules: biogenesis of the broad spectrum antifungal and anti-oomycete compound, oocydin A. J Biol Chem 287 (46):39125-38
- Matsuyama T, Kaneda K, Ishizuka I et al. (1990) Surface-active novel glycolipid and linked 3-hydroxy fatty acids produced by *Serratia rubidaea*. J Bacteriol 172 (6):3015-22

- Matsuyama T, Kaneda K, Nakagawa Y et al. (1992) A novel extracellular cyclic lipopeptide which promotes flagellum-dependent and -independent spreading growth of *Serratia marcescens*. J Bacteriol 174 (6):1769-76
- Matsuyama T, Murakami T, Fujita M et al. (1986) Extracellular Vesicle Formation and Biosurfactant Production by *Serratia marcescens*. J Gen Microbiol 132, 865-875
- Matsuyama T, Sogawa M and Nakagawa Y (1989) Fractal spreading growth of *Serratia marcescens* which produces surface active exolipids. FEMS Microbiol Lett 52 (3):243-6
- Matsuyama T, Fujita M and Yano I (1985) Wetting agent produced by *Serratia marcescens*. FEMS Microbiol Lett 28 (1): 125–29
- Matsuyama T, Tanikawa T and Nakagawa Y (2011) Serrawettins and Other Surfactants Produced by *Serratia*. In Biosurfactants: From Genes to Applications, edited by Gloria Soberón-Chávez, 93-120. Berlin, Heidelberg: Springer Berlin Heidelberg.
- McGowan S, Bycroft B and Salmond G (1998) Bacterial production of carbapenems and clavams: evolution of beta-lactam antibiotic pathways. Trends Microbiol 6 (5):203-8
- McGowan S, Sebahia M, Porter L et al. (1996) Analysis of bacterial carbapenem antibiotic production genes reveals a novel β -lactam biosynthesis pathway. Mol Microbiol 22 (3):415-26
- McGowan S, Sebahia M, Jones S et al. (1995) Carbapenem antibiotic production in *Erwinia carotovora* is regulated by CarR, a homologue of the LuxR transcriptional activator. Microbiology 141 (Pt 3):541-50
- Moellering R, Eliopoulos, G and Sentochnik D (1989) The carbapenems: new broad spectrum beta-lactam antibiotics. J Antimicrob Chemother 24 Suppl A:1-7

- Murdoch S, Trunk K, English G et al. (2011) The opportunistic pathogen *Serratia marcescens* utilizes type VI secretion to target bacterial competitors. *J Bacteriol* 193 (21):6057-69
- Müller H, Westendorf C, Leitner E et al. (2009) Quorum-sensing effects in the antagonistic rhizosphere bacterium *Serratia plymuthica* HRO-C48. *FEMS Microbiol Ecol* 67 (3):468-78
- Müller H, Fürnkranz M, Grube M et al. (2013) Genome Sequence of *Serratia plymuthica* Strain S13, an Endophyte with Germination- and Plant-Growth-Promoting Activity from the Flower of Styrian Oil Pumpkin. *Genome Announc* 1 (4):e00594-13
- Nalini S and Parthasarathi R (2014) Production and characterization of rhamnolipids produced by *Serratia rubidaea* SNAU02 under solid-state fermentation and its application as biocontrol agent. *Bioresour Technol* 173:231-38
- Newman D and Cragg G (2007) Natural products as sources of new drugs over the last 25 years. *J Nat Prod* 70 (3):461-77
- Nishida M, Mine Y, Nonoyama S et al. (1977) Nocardicin A, a new monocyclic beta-lactam antibiotic III. In vitro evaluation. *J Antibiot (Tokyo)* 30 (11):917-25
- Niveshika E, Mishra A, Singh A et al. (2016) Structural Elucidation and Molecular Docking of a Novel Antibiotic Compound from Cyanobacterium *Nostoc* sp. MGL001. *Front Microbiol* 7:1899
- Overhage J, Bains M, Brazas M et al. (2008) Swarming of *Pseudomonas aeruginosa* is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. *J Bacteriol* 190 (8):2671-9
- Perry J (1961) Prodigiosin in an Actinomycete. *Nature* 191: 77–8

- Piel J (2010) Biosynthesis of polyketides by trans-AT polyketide synthases. *Nat Prod Rep* 27 (7):996-1047
- Pradel E, Zhang Y, Pujol N et al. (2007) Detection and avoidance of a natural product from the pathogenic bacterium *Serratia marcescens* by *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 104 (7):2295-300
- Pérez-Tomás R, Montaner B, Llagostera E et al. (2003) The prodigiosins, proapoptotic drugs with anticancer properties. *Biochem Pharmacol* 66 (8):1447-52
- Schmidt R, Jager V, Zuhlke D et al. (2017) Fungal volatile compounds induce production of the secondary metabolite Sodorifen in *Serratia plymuthica* PRI-2C. *Sci Rep* 7 (1):862
- Singh R and Jha P (2016) The Multifarious PGPR *Serratia marcescens* CDP-13 Augments Induced Systemic Resistance and Enhanced Salinity Tolerance of Wheat (*Triticum aestivum* L.). *PLoS One* 11 (6):e0155026
- Slater H, Crow M, Everson L et al. (2003) Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in *Serratia* via both quorum-sensing-dependent and -independent pathways. *Mol Microbiol* 47 (2):303-20
- Soenens A (2018) Characterisation of Non-symbiotic Rhizobial and *Serratia* Populations from Soil. PhD Thesis, Universidad Politécnica de Madrid. <https://www.educacion.es/teseo/mostrarRef.do?ref=1664382>
- Soberón-Chávez G and Maier R (2011) Biosurfactants: A General Overview. In *Biosurfactants: From Genes to Applications*, edited by Gloria Soberón-Chávez, 1-11. Berlin, Heidelberg: Springer Berlin Heidelberg
- Solé M, Rius N, Francia A et al. (1994) The effect of pH on prodigiosin production by non-proliferating cells of *Serratia marcescens*. *Lett Appl Microbiol* 19 (5):341-4

- Soo P, Horng Y, Chang Y et al. (2014) ManA is regulated by RssAB signaling and promotes motility in *Serratia marcescens*. Res Microbiol 165 (1):21-9
- Soto-Cerrato V, Montaner B, Martinell M et al. (2005) Cell cycle arrest and proapoptotic effects of the anticancer cyclodepsipeptide serratamolide (AT514) are independent of p53 status in breast cancer cells. Biochem Pharmacol 71 (1-2):32-41
- Srobel G, Li JY, Sugawara F et al. (1999) Oocydin A, a chlorinated macrocyclic lactone with potent anti-oomycete activity from *Serratia marcescens*. Microbiology 145 (12):3557-64
- Stankovic N, Senerovic L, Ilic-Tomic T et al. (2014) Properties and applications of undecylprodigiosin and other bacterial prodigiosins. Appl Microbiol Biotechnol 98 (9):3841-58
- Su C, Xiang Z, Liu Y et al. (2016) Analysis of the genomic sequences and metabolites of *Serratia surfactantifaciens* sp. nov. YD25. BMC Genomics 17 (1):865
- Sun X, Shen X, Jain R et al. (2015) Synthesis of chemicals by metabolic engineering of microbes. Chem Soc Rev 44 (11):3760-85
- Suryawanshi R, Patil C, Koli S et al. (2017) Antimicrobial activity of prodigiosin is attributable to plasma-membrane damage. Nat Prod Res 31 (5):572-7
- Talarico T and Dobrogosz W (1989) Chemical characterization of an antimicrobial substance produced by *Lactobacillus reuteri*. Antimicrob Agents Chemother 33 (5):674-9
- Tanikawa T, Nakagawa Y and Matsuyama T (2006) Transcriptional downregulator hexS controlling prodigiosin and serrawettin W1 biosynthesis in *Serratia marcescens*. Microbiol Immunol 50 (8):587-96

- Thomson N, Crow M, McGowan S et al. (2000) Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* is under quorum sensing control. *Mol Microbiol* 36:539-56
- Till M and Race P (2014) Progress challenges and opportunities for the re-engineering of trans-AT polyketide synthases. *Biotechnol Lett* 36 (5):877-88
- Trunk K, Peltier J, Liu Y et al. (2018) The type VI secretion system deploys antifungal effectors against microbial competitors. *Nat Microbiol* 3 (8):920-31
- Varivarn K, Champa L, Silby M et al. (2013) Colonization strategies of *Pseudomonas fluorescens* Pf0-1: activation of soil-specific genes important for diverse and specific environments. *BMC Microbiol* 13:92
- von Reuss S, Kai M, Piechulla B et al. (2010) Octamethylbicyclo[3.2.1]octadienes from the rhizobacterium *Serratia odorifera*. *Angew Chem Int Ed Engl* 49 (11):2009-10
- Wasserman H, Keggi J and McKeon J (1961) Serratamolide, a metabolic product of *Serratia*. *J Am Chem Soc* 83 (19):4107-8
- Wasserman H, Keggi J and McKeon J (1962) The Structure of Serratamolide1-3. *J Am Chem Soc* 84 (15):2978-82
- Weber T, Blin K, Duddela S et al. (2015) antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Res* 43 (W1):W237-W243
- Weise T, Thurmer A, Brady S et al. (2014) VOC emission of various *Serratia* species and isolates and genome analysis of *Serratia plymuthica* 4Rx13. *FEMS Microbiol Lett* 352 (1):45-53
- Wenke K, Kai M and Piechulla B (2010) Belowground volatiles facilitate interactions between plant roots and soil organisms. *Planta* 231 (3):499-506

- Wenke K, Weise T, Warnke R et al. (2012) Bacterial Volatiles Mediating Information Between Bacteria and Plants. In Biocommunication of Plants, edited by Günther Witzany and František Baluška, 327-347. Berlin, Heidelberg: Springer Berlin Heidelberg
- Williams R and Qadri S (1980) The pigment of *Serratia*. In the Genus *Serratia*. Edited by A. von Graevenitz & S.J Rubin. Boca Raton, Florida: CRC Press
- Williams R, Gott C and Qadri S (1971) Induction of pigmentation in nonproliferating cells of *Serratia marcescens* by addition of single amino acids. J Bacteriol 106 (2):444-8
- Williams R (1973) Biosynthesis of Prodigiosin, a Secondary Metabolite of *Serratia marcescens*. Appl Microbiol 25(3): 396–402
- Yamaguchi H, Nakayama Y, Takeda K et al. (1957) A new antibiotic, althiomycin. J Antibiot (Tokyo) 10 (5):195-200

Table 1. Biocontrol activities described in *Serratia*

Strains	Biocontrol activity	Reference
<i>S. marcescens</i> NBRI 1213	Biologic control of foot and root rot of <i>Piper betle</i> caused by the oomycete <i>Phytophthora nicotianae</i>	(Lavania et al. 2006)
<i>S. marcescens</i> CDP-13	Suppressed fungal pathogens in wheat	(Singh and Jha 2016)
<i>S. plymuthica</i> HRO-C48	Suppression of <i>Verticillium dahliae</i> in strawberry, oilseed rape and olive, and pathogen <i>Rhizoctonia solani</i> on lettuce. Production mVOCs negatively influence the mycelial growth of <i>R. solani</i>	(Kurze et al. 2001; Grosch et al. 2005; Kai et al. 2007; Müller et al. 2009)
<i>S. plymuthica</i> S13	Antagonist toward <i>Didymella bryoniae</i> , the causal agent of black rot in pumpkins under field conditions	(Fürnkranz et al. 2012; Müller et al. 2013)
<i>S. plymuthica</i> 4Rx13	Antifungal activities, mVOCs (sodorifen)	(Kai et al. 2007; Weise et al. 2014; Domik et al. 2016a)
<i>S. proteamaculans</i> 1-102 (1-102)	Biocontrol against the fungal pathogen <i>V. dahliae</i>	(Alström 2001)

mVCOs: microbial Volatile Organic Compounds.

Table 2. Genomic features of *Serratia* sp. B1_6

Strain	Number of contigs	Largest contig (bp)	Total genome length (bp)	G+C (%)	N50
B1_6	19	2,731,268	5,525,162	56.07	533,702

Table 3. Presence of antibiotic, antifungal and mVOCs gene clusters in *Serratia* sp. B1_6 genome and comparison with phenotypic screening results

Strain	Antibiotic production		Antifungal		mVOCs	
	Carbapenem biosynthetic cluster	<i>E. coli</i> ESS screening	Oocydin A biosynthetic cluster	Screening against <i>V. dahliae</i>	Sodorifen biosynthetic cluster	Terpene antiSMASH prediction
B1_6	no	++	Yes	+++	yes	+

- Negative, <+ some activity, +activity, ++ high activity, +++ very high activity and ++++ great activity.

Prodigiosin
Williamson et al. 2005

Carbapenem
Coulthurst et al. 2005

Sodorifen
Domik et al. 2016

Oocydin A
Matilla et al. 2012

Althiomycin
Gerc et al. 2012

Serrawettin W1
Matsuyama et al. 2011

Serrawettin W2
Matsuyama et al. 2011

Rubiwettin R1
(Matsuyama et al. 1990,
Clements et al. 2019)

Rubiwettin RG1
(Matsuyama et al. 1990,
Clements et al. 2019)

Figure 2. *Serratia* sp. B1_6 antifungal and antibiotic production screening results